

A Gene for Autosomal Dominant Hypohidrotic Ectodermal Dysplasia (*EDA3*) Maps to Chromosome 2q11-q13

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Summary

Autosomal dominant hypohidrotic ectodermal dysplasia (ADHED) is a disorder characterized by fine, slow-growing scalp and body hair, sparse eyebrows and eyelashes, decreased sweating, hypodontia, and nail anomalies. By genetic linkage analysis of a large ADHED kindred, we have mapped a gene for ADHED (*EDA3*) to the proximal long arm of chromosome 2 (q11-q13). Obligate recombinations localize *EDA3* to an ~9-cM interval between D2S1321 and D2S308, with no apparent recombinations with markers D2S1343, D2S436, D2S293, D2S1894, D2S1784, D2S1890, D2S274, and CHLC.GAAT11C03.

Introduction

Ectodermal dysplasias (ED) are a heterogeneous group of disorders characterized by developmental dystrophies of a wide array of ectodermal structures, most frequently involving the skin and sweat glands (hypohidrosis), hair (trichodysplasia), nails (onychodysplasia), and teeth (hypodontia or anodontia). About 175 clinically and genetically different forms of ED have been cataloged (Freire-Maia and Pinheiro 1984, 1987; Gorlin et al. 1970; <http://www.ncbi.nlm.nih.gov/Omim>), with X-linked recessive, X-linked dominant, autosomal recessive, and autosomal dominant patterns of inheritance observed. However, only a small number of ED genes have been genetically mapped or cloned.

We recently described a novel form of autosomal dominant hypohidrotic ED (ADHED) in 38 individuals over six generations in one large kindred (Aswegan et al.

1997). The principal clinical features of this form of ADHED are smooth, dry, thin skin (78%); sparse, fine, slow-growing hair (89%); sparse eyebrows (96%), eyelashes (100%), and body hair (62%); decreased sweating (85%) and heat intolerance (50%); onychodysplasia (39%); and hypodontia, anodontia, microdontia, or other dental anomalies (100%). Here, we report genetic linkage analysis of this ADHED family and localization of the *EDA3* locus to an ~9-cM interval on proximal chromosome 2q (q11-q13), an interval spanned by a single large YAC contig.

Subjects and Methods

Pedigree and Genotyping

As shown in figure 1, we obtained blood samples from 21 affected and 16 unaffected members of the ADHED kindred with informed consent. Power modeling analyses were carried out using SLINK, on the assumption that ADHED in this kindred is a fully dominant trait with a frequency of 10^{-5} and using polymorphic markers with five equally frequent alleles. DNA was prepared by standard methods, and genotyping was done by PCR using ³²P]-radiolabeled primers for polymorphic microsatellite markers from the Research Genetics Screening Set 8. Two-point linkage analyses were carried out using MLINK from the LINKAGE 5.1 program package under the assumption that ADHED is a fully dominant trait with a frequency of 10^{-5} and using marker allele frequencies taken from the Marshfield Medical Foundation database (<http://www.marshfield.org/genetics/>) or the Genome Database (<http://www.gdb.org/>). Primer pairs for additional microsatellite markers were purchased from Research Genetics.

Results and Discussion

Exclusion of Linkage to Known Autosomal Dominant ED Loci

When this study was undertaken, three other autosomal dominant ED loci had been mapped: that for Clouston ED (*EDA2*; MIM 129500) to the pericentric

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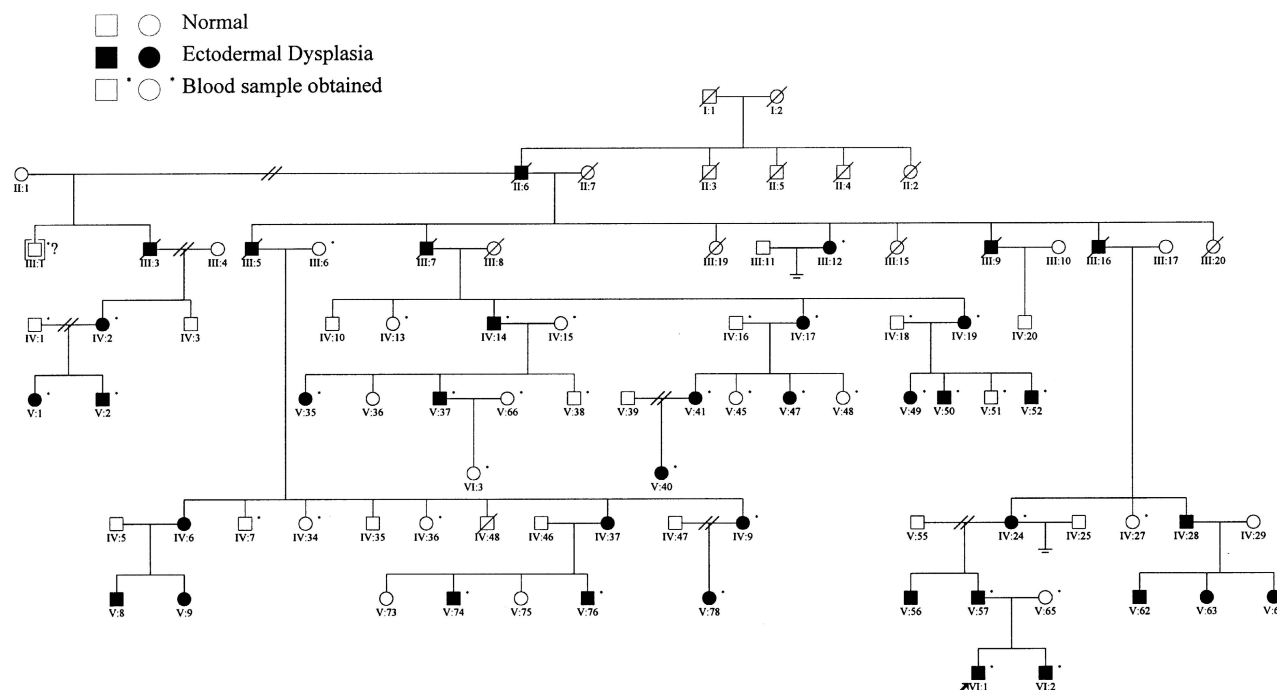


Figure 1 Pedigree of ADHED kindred, after Aswegan et al. (1997). Numbering is not consecutive in generations, because of ascertainment of relatives at different times and the need to keep numbering consistent. Asterisks (*) denote individuals sampled for this study.

region of chromosome 13q (Kibar et al. 1996), for ectrodactyly, ectodermal dysplasia (EEC) syndrome (*EEC*; MIM 129900) to 7q11.2-q21.3 (Hasegawa et al. 1991; Qumsiyeh 1992), and for split hand/split foot malformation (*SHFD1*; MIM 183600) to 7q21.2-q21.3 (Crackower et al. 1996). The phenotypes of these disorders are very different from that of ADHED, making it quite unlikely that ADHED might be allelic to Clouston ED, EEC, or SHFM. Nevertheless, it was crucial that we first exclude these possibilities. The Clouston *EDA2* locus has shown no recombination with microsatellite markers D13S175 and D13S141 (Kibar et al. 1996). Power modeling analysis of the ADHED kindred using SLINK yielded hypothetical LOD scores of 7.11 at $\theta = 0$, 5.88 at $\theta = .10$, 4.53 at $\theta = .20$, 3.07 at $\theta = .30$, and 1.46 at $\theta = .40$. However, D13S175 yielded a maximum LOD score (Z_{\max}) in the ADHED kindred of only .32 at $\theta = .50$, and D13S141 yielded a maximum LOD score of 0 at $\theta = .50$. These data thus are not indicative of allelism between ADHED and Clouston ED.

Similarly, both *SHFM1* and *EEC1* have shown no recombination with marker D7S821 (Crackower et al. 1996). However, linkage analysis of D7S821 in the ADHED kindred yielded a Z_{\max} of 0 at $\theta = .50$, again not indicative of allelism between ADHED and EEC syndrome. A second EEC locus (*EEC2*; MIM 602077) was recently mapped to chromosome 19 (O'Quinn et al.

1997), but this report appeared subsequent to our mapping of *EDA3* and so was not considered.

Localization of the *EDA3* ADHED Locus to Chromosome 2

A total of 194 microsatellite markers randomly distributed on all chromosomes were analyzed in the ADHED kindred by pairwise linkage analysis before marker D2S410, located close to the centromere on proximal chromosome 2q, yielded a Z_{\max} of 3.93 at $\theta = 0$. Subsequent analysis of additional flanking markers from Screening Set 8 yielded significant positive LOD scores for D2S2972 ($Z_{\max} = 5.47$ at $\theta = 0$), D2S442 ($Z_{\max} = 3.03$ at $\theta = 0$), D2S1790 ($Z_{\max} = 4.06$ at $\theta = .08$), and D2S1777 ($Z_{\max} = 3.00$ at $\theta = .06$). Consequently, additional markers from this region were identified from the Marshfield chromosome 2 linkage map (<http://www.marshfield.org/genetics/>), and two-point linkage analysis of ≥ 18 of these markers confirmed linkage of ADHED in this kindred to this region of chromosome 2, with the highest Z_{\max} obtained with D2S436 (LOD 7.82 at $\theta = 0$) (table 1). In fact, no obligatory recombinants were observed with eight markers in this region, and Z_{\max} values >3.0 were found spanning at least 26.3 cM. This rather extensive distribution of significantly positive LOD scores was unexpected and suggested the

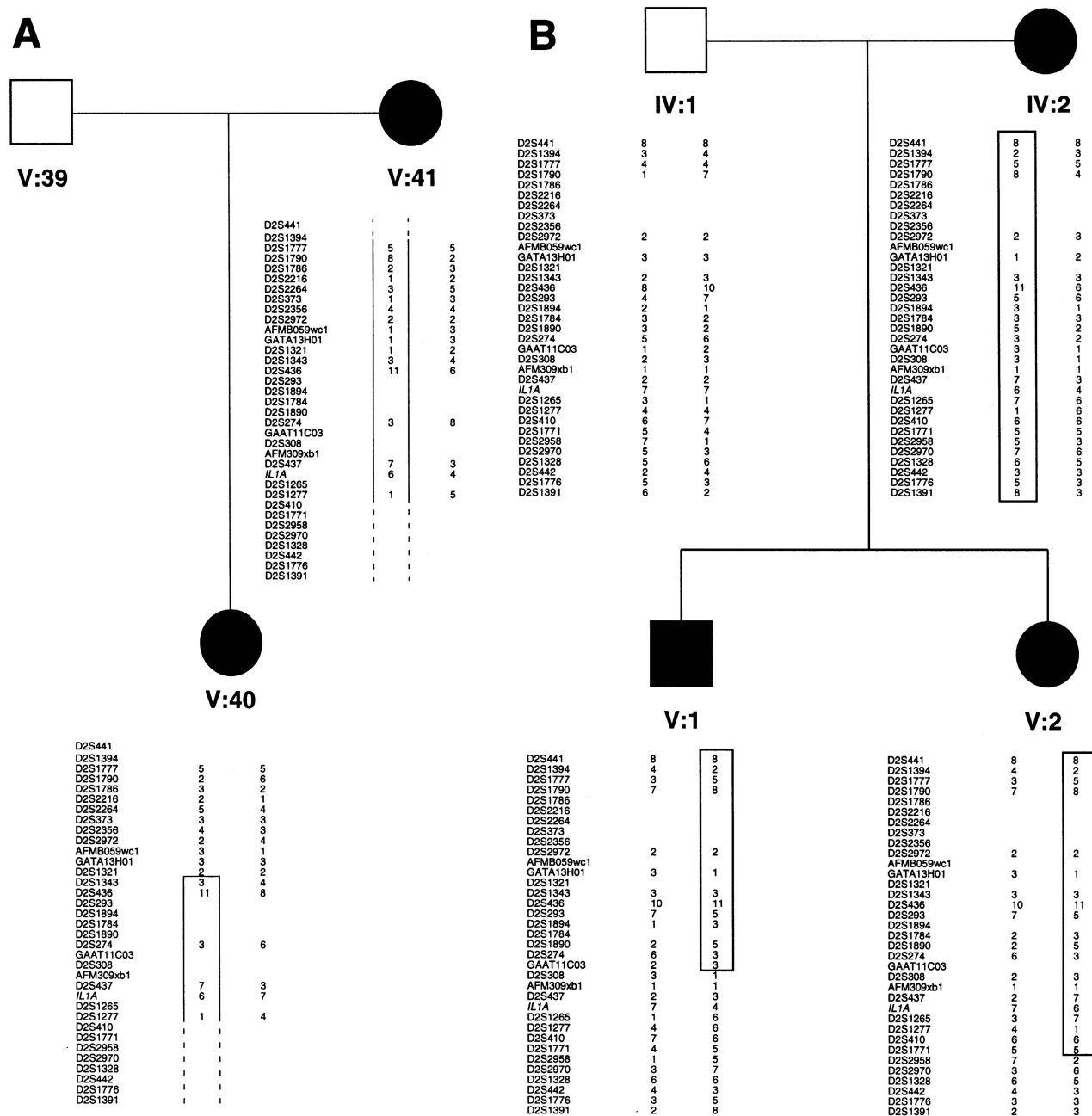


Figure 2 Proximal chromosome 2q marker haplotypes in critical members of the ADHED kindred. A, Portion of the kindred with an apparent recombinant between *EDA3* and proximal flanking marker D2S1321 (individual V:40); individual V:39 was not available. B, Portion of the kindred with apparent recombinant between *EDA3* and distal flanking marker D2S308 (individual V:1). Boxes denote haplotypes or portions of haplotypes inferred on the basis of analyses of other family members as being ancestrally related to ADHED (e.g., individual IV: 2). Alleles as described in the Genome Database when given in that resource (<http://www.gdb.org/>). Marker order is our best reconciliation of the Marshfield sex-averaged chromosome 2 linkage map (<http://www.marshfield.org/genetics/>) and the Whitehead YAC contig STS-content physical map (http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map), with the position of D2S437 moved proximally relative to the Marshfield genetic map to maximize parsimony with our linkage data.

Table 1**Two-Point LOD Scores for ADHED and Chromosome 2 Markers**

Marker	Z_{\max}	θ	cM ^a
D2S441	.27	.29	84.3
D2S1394	2.14	.10	88.3
D2S1777	3.00	.06	96.9
D2S1790	4.06	.08	100.6
D2S2972	5.47	.00	111.9
D2S1343	1.33	.00	113.0
D2S274	3.72	.00	113.0
CHLC.GATA13H01	5.49	.00	113.5
D2S436	7.82	.00	115.6
D2S293	4.61	.00	115.6
D2S1890	7.02	.00	116.7
D2S1784	2.34	.00	116.7
<i>IL1A</i>	4.34	.04	120.5
D2S1265	4.12	.05	121.5
D2S1277	4.16	.08	121.5
D2S437	1.88	.08	122.7
D2S410	3.93	.00	122.7
D2S1771	3.83	.05	123.2
D2S2970	2.19	.11	124.7
D2S2958	4.04	.07	129.5
D2S1328	.06	.11	131.1
D2S442	3.03	.00	146.0
D2S1776	1.02	.19	171.6
D2S1391	.01	.50	184.8

^a Kosambi cM and marker order are from the Marshfield sex-averaged chromosome 2 linkage map (<http://www.marshfield.org/genetics/>).

possibility of suppressed recombination on the ADHED chromosome in the region of the *EDA3* gene. The most likely cause of suppressed recombination might be an inversion on the mutant chromosome in this region; however, high-resolution banded chromosome analysis of one affected member of the ADHED kindred showed a normal 46,XY karyotype, with no visible inversion of chromosome 2.

Obligate recombinations between *EDA3* and proximal marker D2S1790 (individuals V:38, V:40, and V:50) and distal marker *IL1A* (individual V.1) defined the preliminary maximal genetic interval for *EDA3* as a 19.9-cM region on the Marshfield sex-averaged map of chromosome 2 (<http://www.marshfield.org/genetics/>). This genomic region is spanned by five YAC contigs (with four gaps) in the Whitehead physical map of chromosome 2: WC2.5, WC2.6, WC2.7, WC2.8, and WC2.9 (http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map). Comparison of the Marshfield genetic map and the Whitehead physical YAC contig sequence-tagged site (STS) content map revealed a number of discrepancies of marker order throughout this region, the most significant of which involved exclusion of distal flanking marker *IL1A* from the distal-most YAC contig spanning the region, WC2.9, although marker D2S437, which was also recombinant with *EDA3*, is located within the WC2.9 contig. Our subsequent analyses were completely

consistent with marker order in the Whitehead physical STS content map, though not with the Marshfield genetic map, and because the marker order in the physical STS content map seems more likely to be correct than that in the genetic map, we considered the corrected preliminary *EDA3* maximal interval to be defined proximally by D2S1790 and distally by D2S437.

To more closely delimit the *EDA3* interval, we used the Whitehead STS content map of the region to select additional markers for genetic analysis, most of which were typed only in selected members of the ADHED kindred. As shown in figure 2, we were able to derive unambiguous recombinant haplotypes that defined the proximal flanking boundary of the *EDA3* region at D2S1321 (individual V:40; fig. 2A) and the distal flanking boundary at D2S308 (individual V:1; fig. 2B). Both of these flanking markers are located within Whitehead YAC contig WC2.9 and define a genetic interval estimated to be ~9 cM (http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map).

It may be that the *EDA3* genetic interval can be reduced further by analysis of additional ADHED families. Jorgensen (1974) and Jorgensen et al. (1987; OMIM 129200, 129490), for example, described two ADHED families with phenotypic manifestations somewhat similar to that in the present kindred, although we cannot know a priori whether these disorders are allelic to *EDA3* and thus whether they would be useful. The National Council for Biotechnology Information Gene Map of the Human Genome (<http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/chr?2>) lists ~10 defined genes and 39 anonymous expressed sequence tags assigned to the *EDA3* interval, none of which seem to be strong candidates for *EDA3*, and none of which exhibit homology to the predicted protein product of the gene for X-linked recessive hypohidrotic ED (*EDA1*; OMIM 305100; Kere et al. 1996). Moreover, homology between the human and mouse genomes is not entirely clear in this region, with areas of synteny with both mouse chromosomes 1 and 2 but with no evident murine homologues to ADHED in either case. It thus seems likely that the best approach to eventual identification of the *EDA3* gene will likely be analysis of candidate genes in the region for pathological mutations.

Acknowledgments

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